

Fig. 1

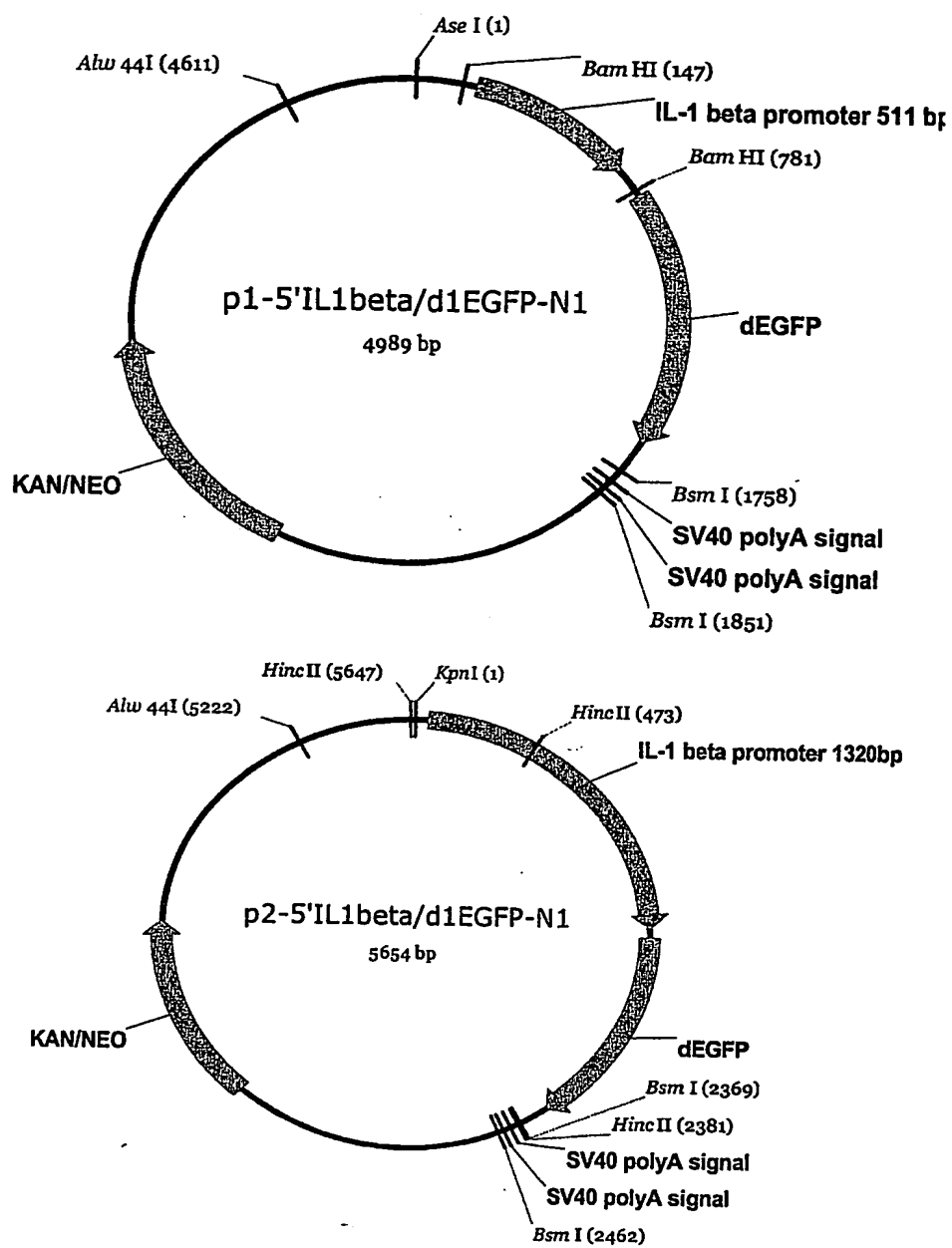


Fig. 2

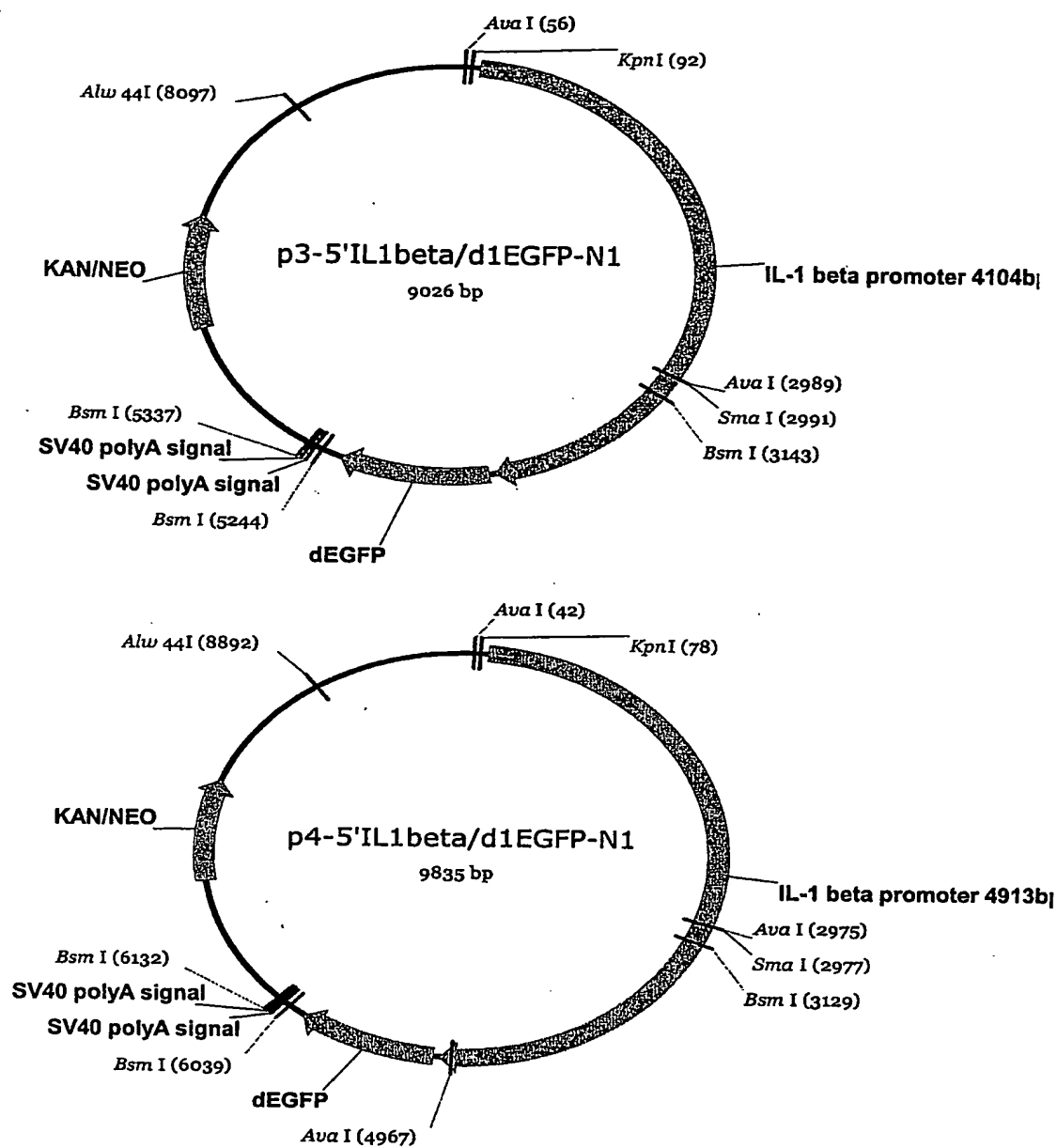


Fig. 3

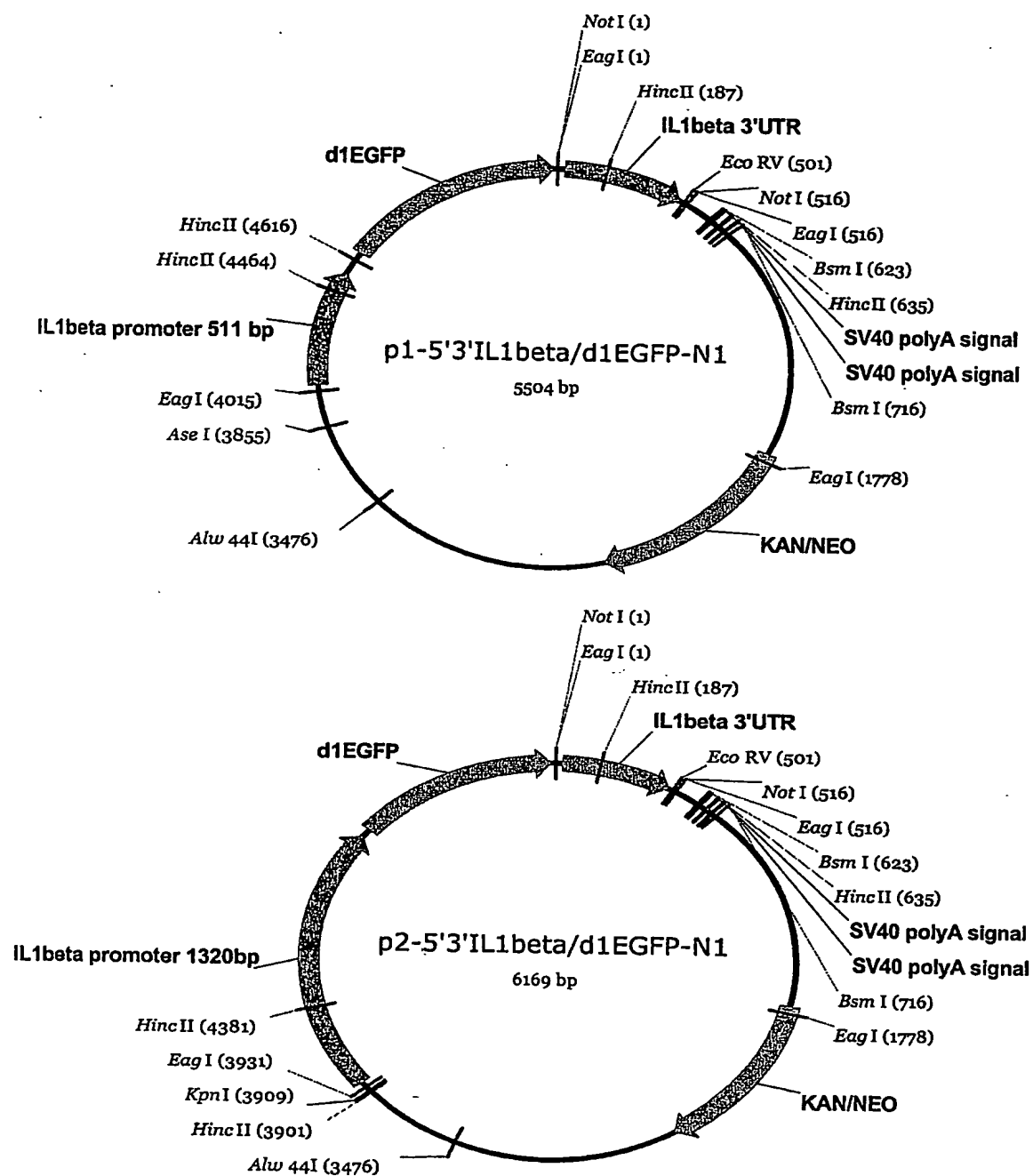


Fig. 4

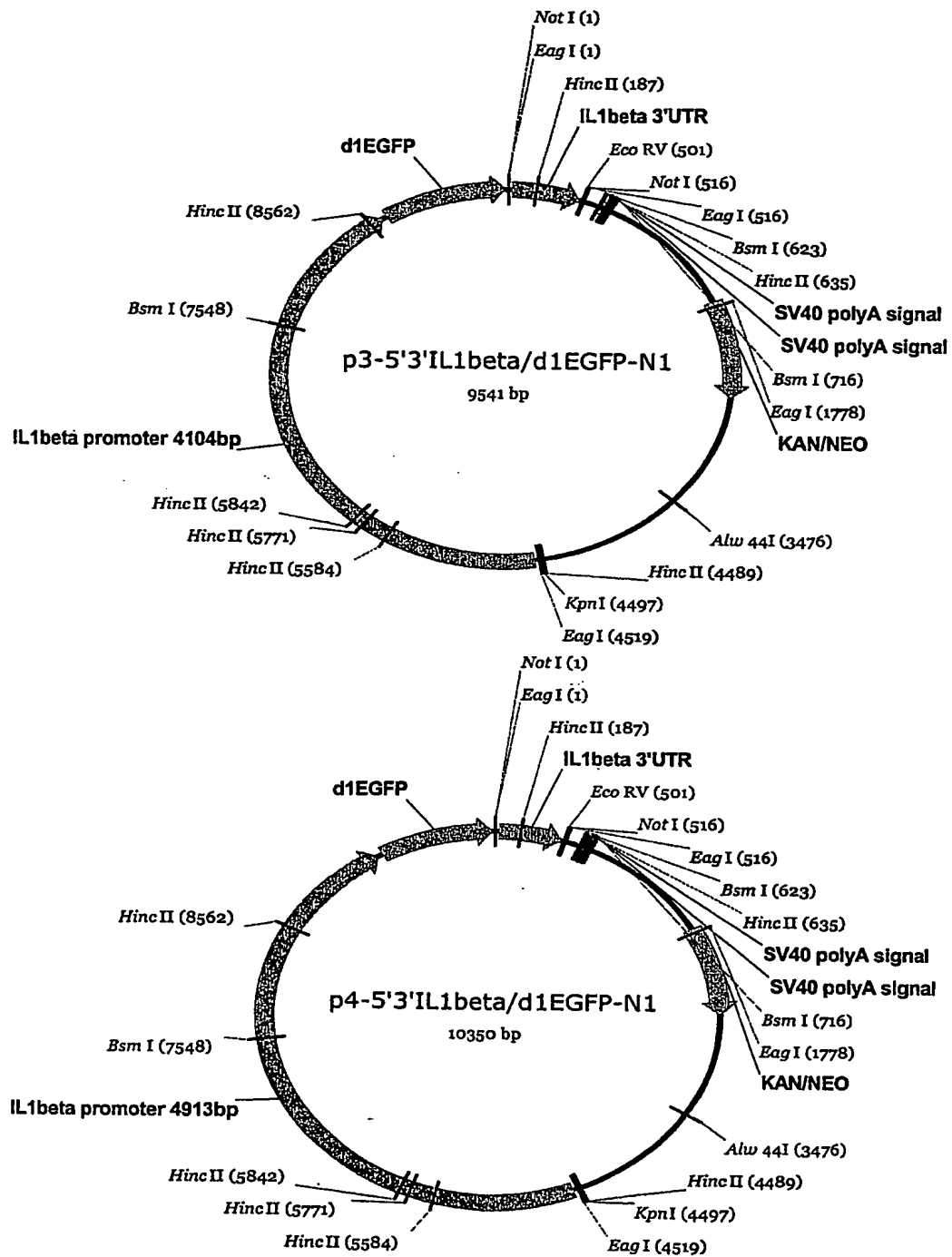


Fig. 5

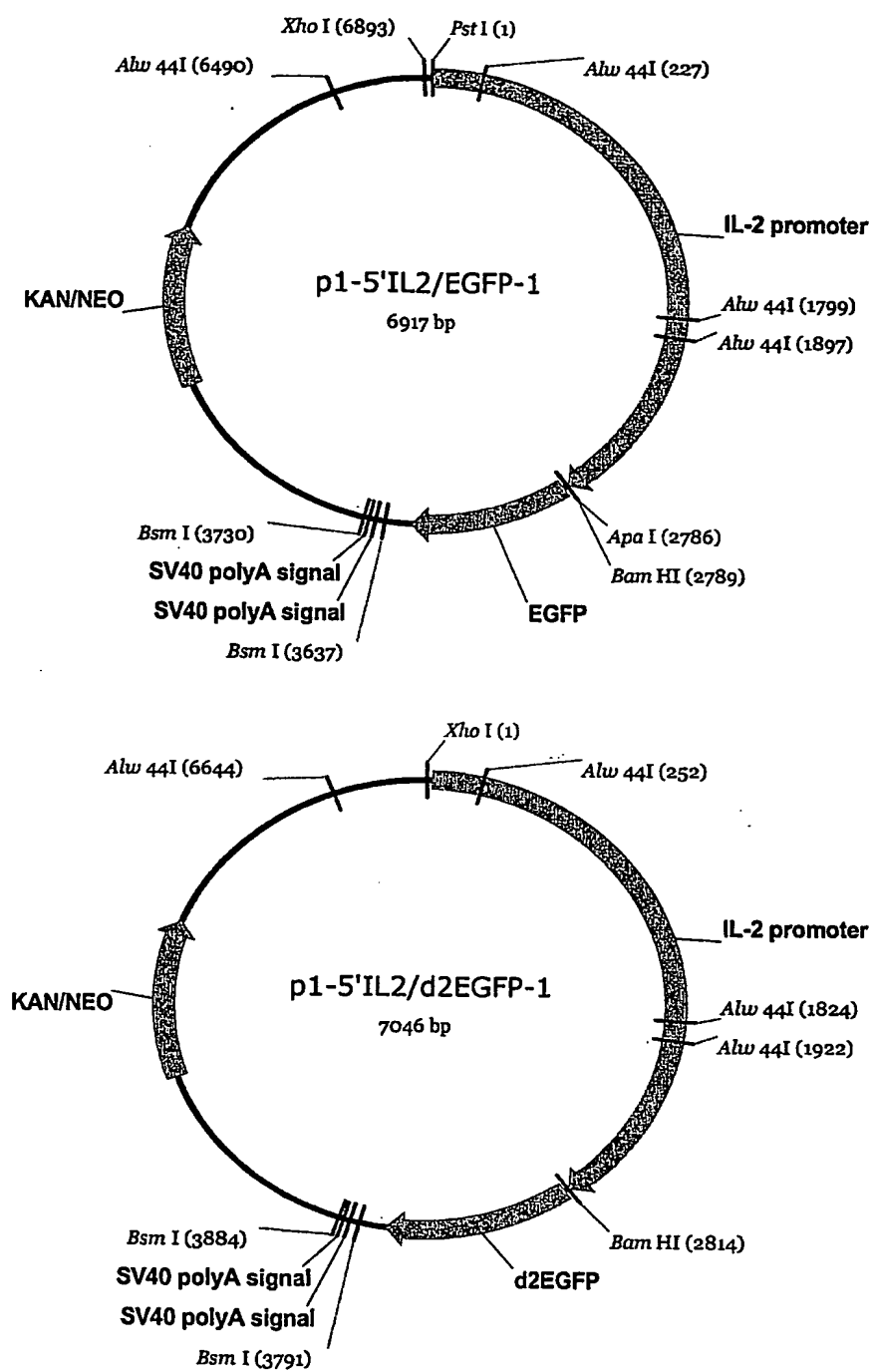


Fig. 6

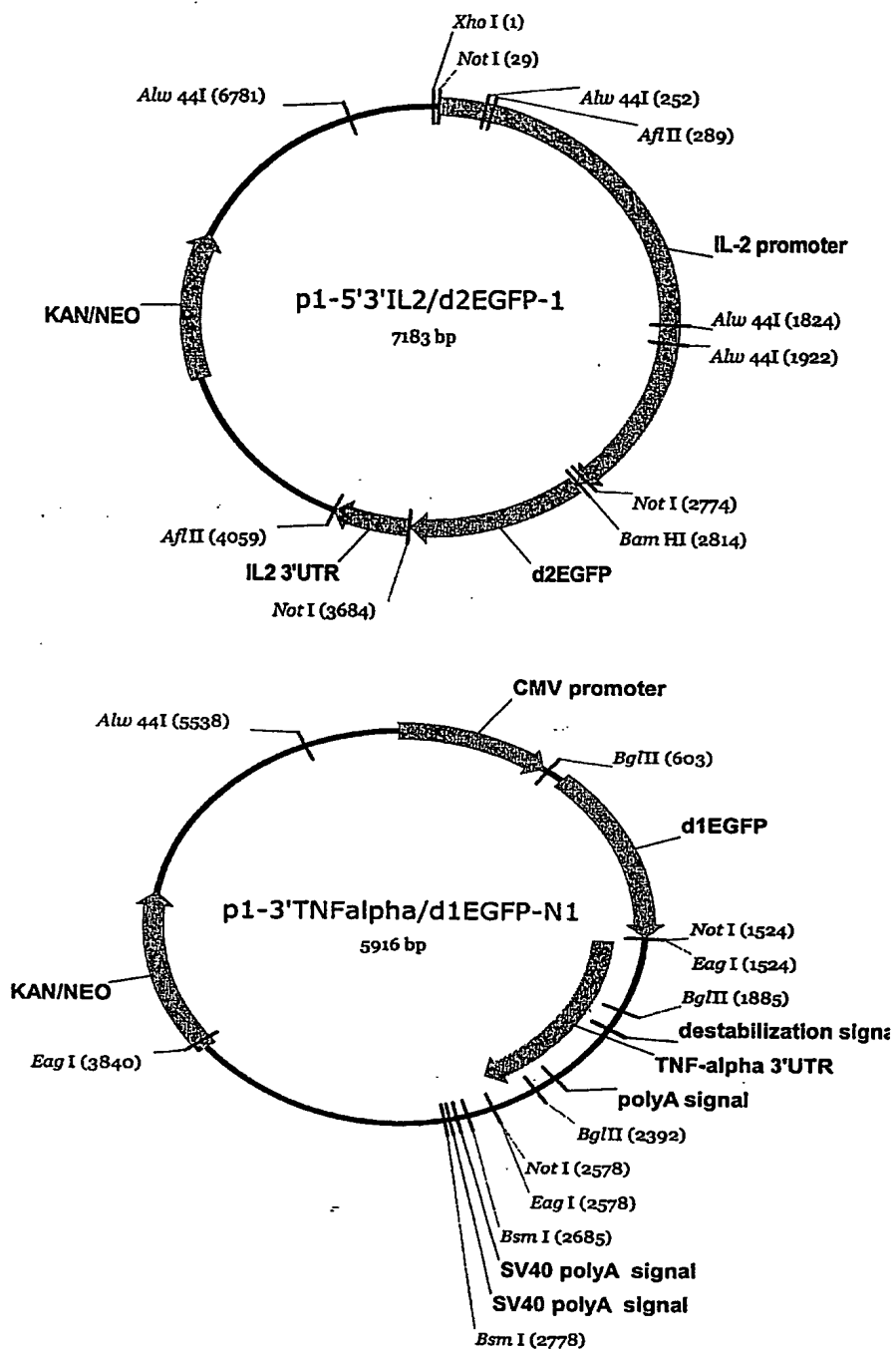
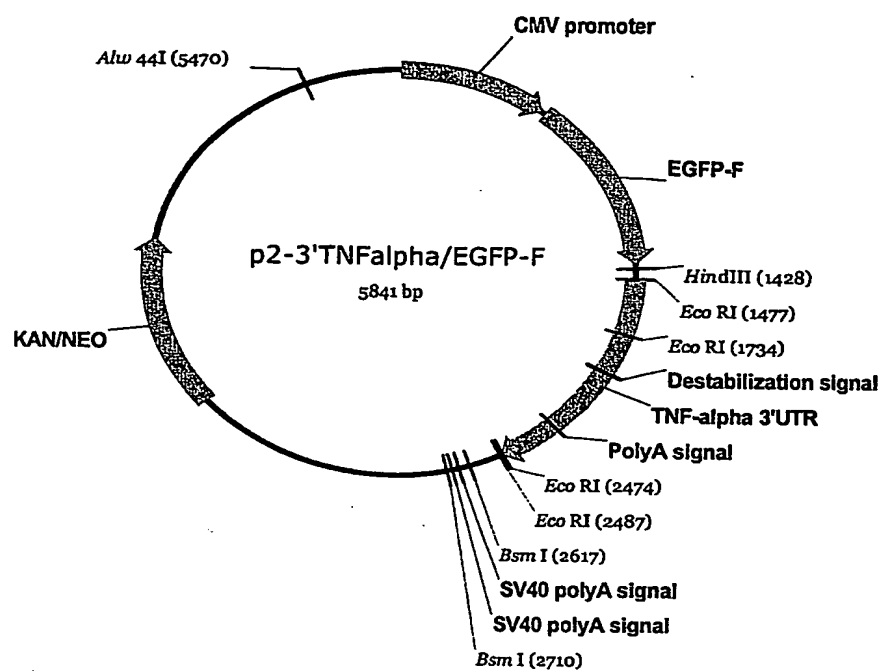
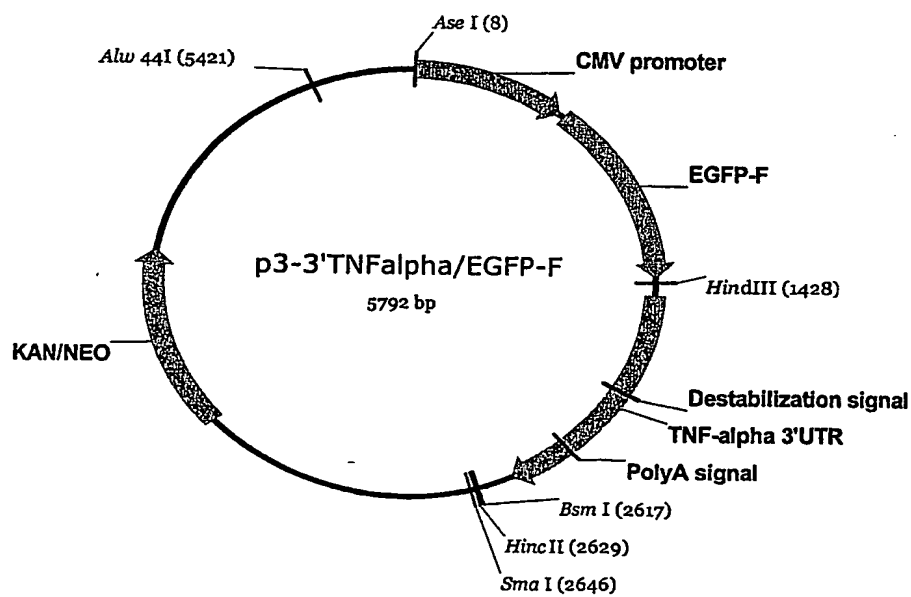
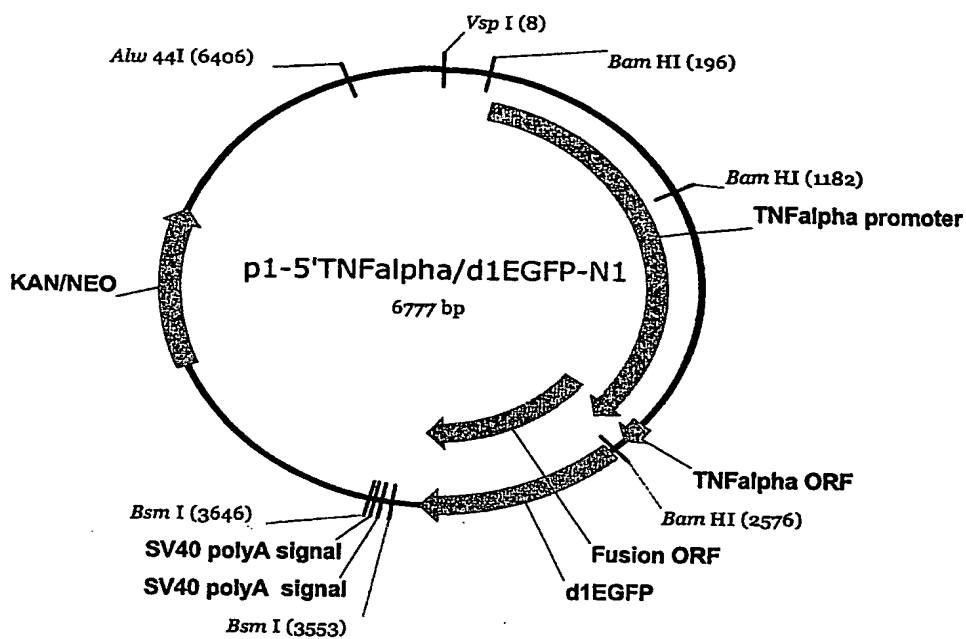
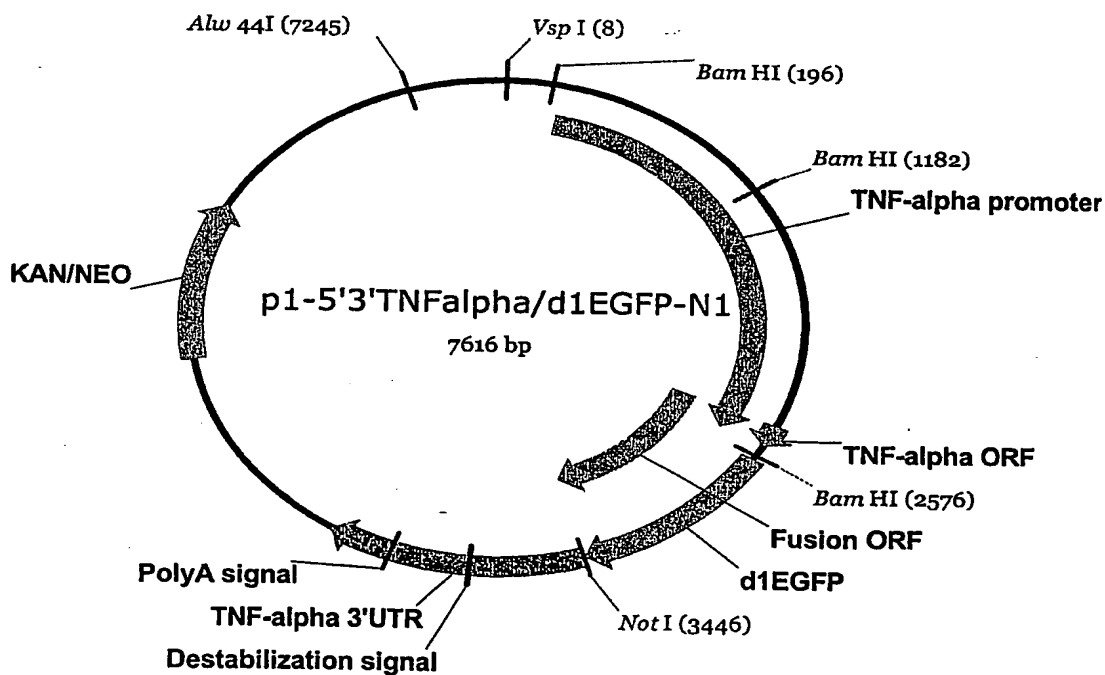


Fig. 7

**Fig. 8****Fig. 9**

**Fig. 10****Fig. 11**

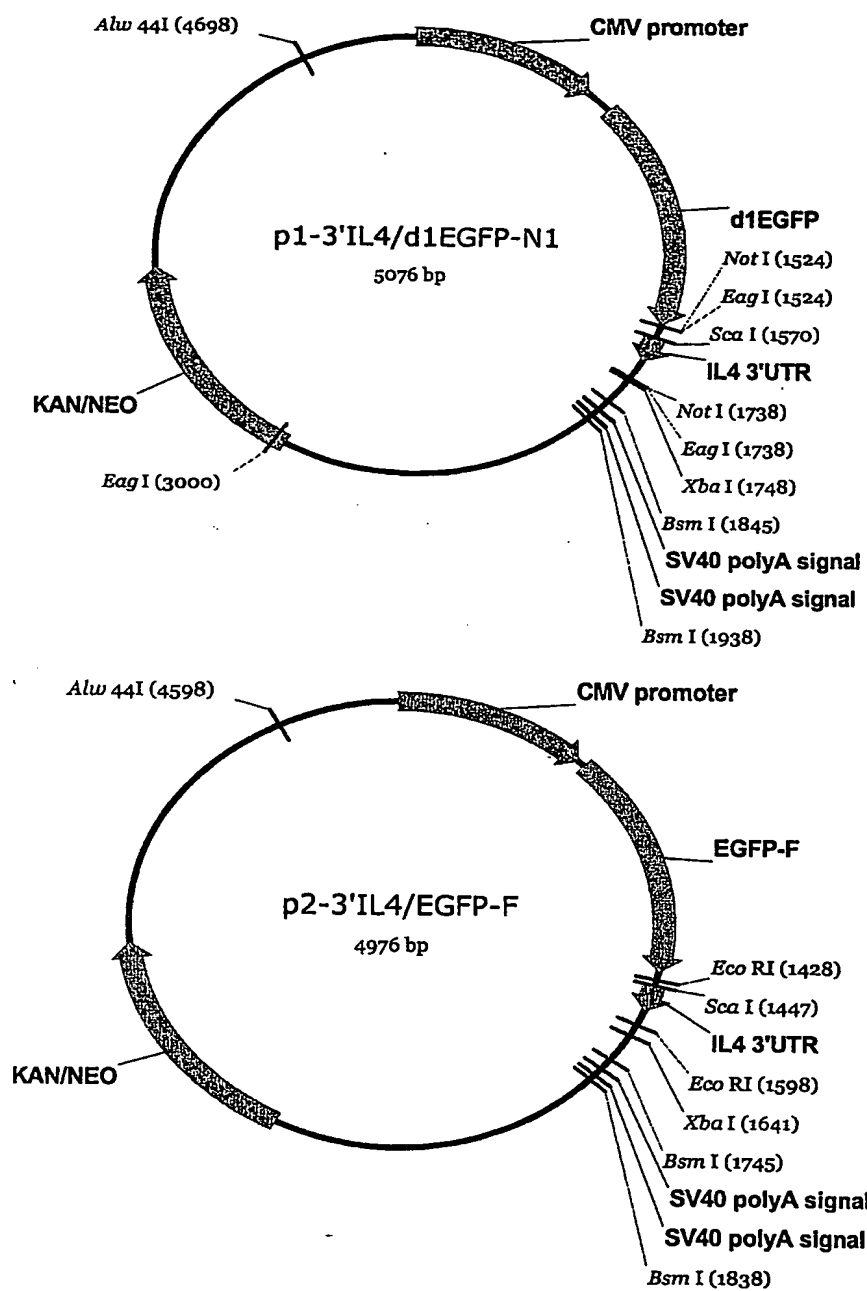
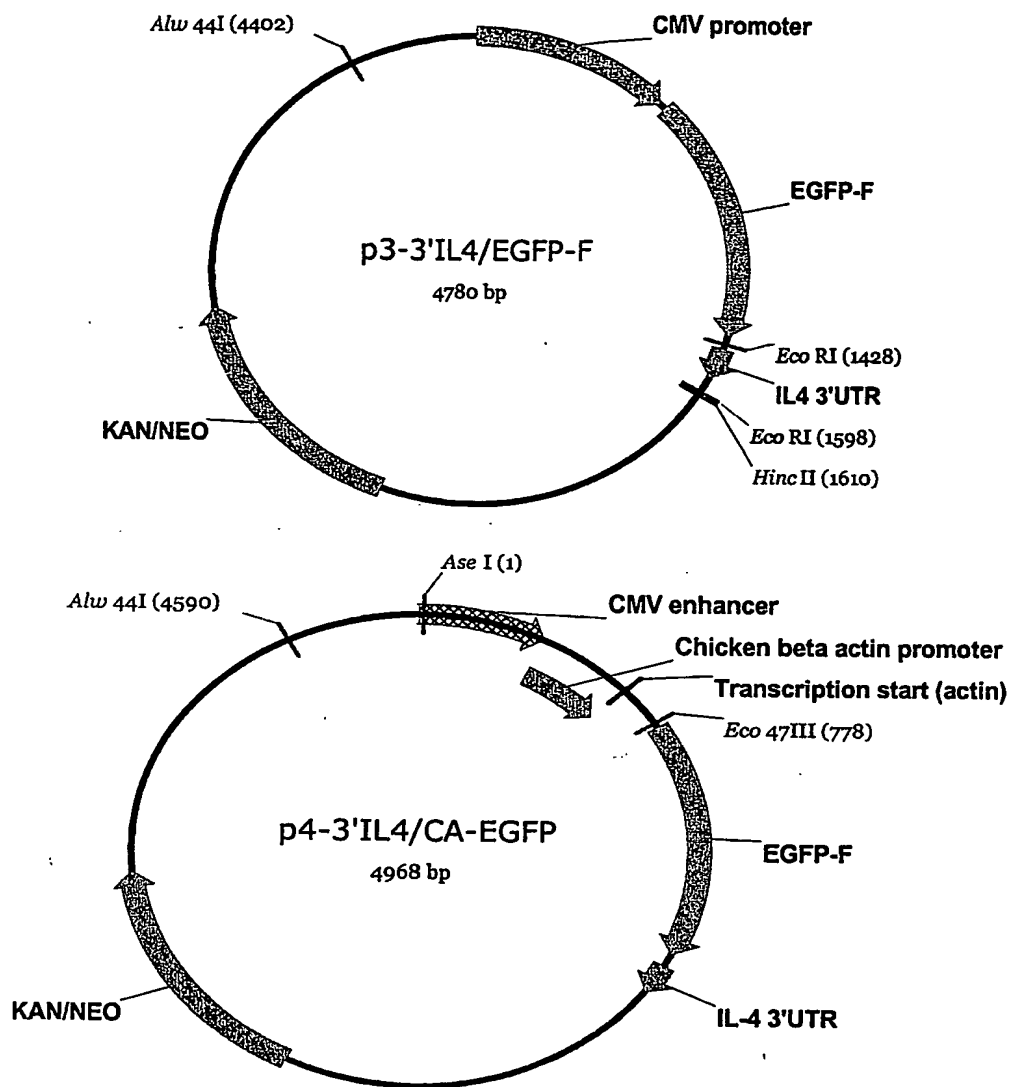
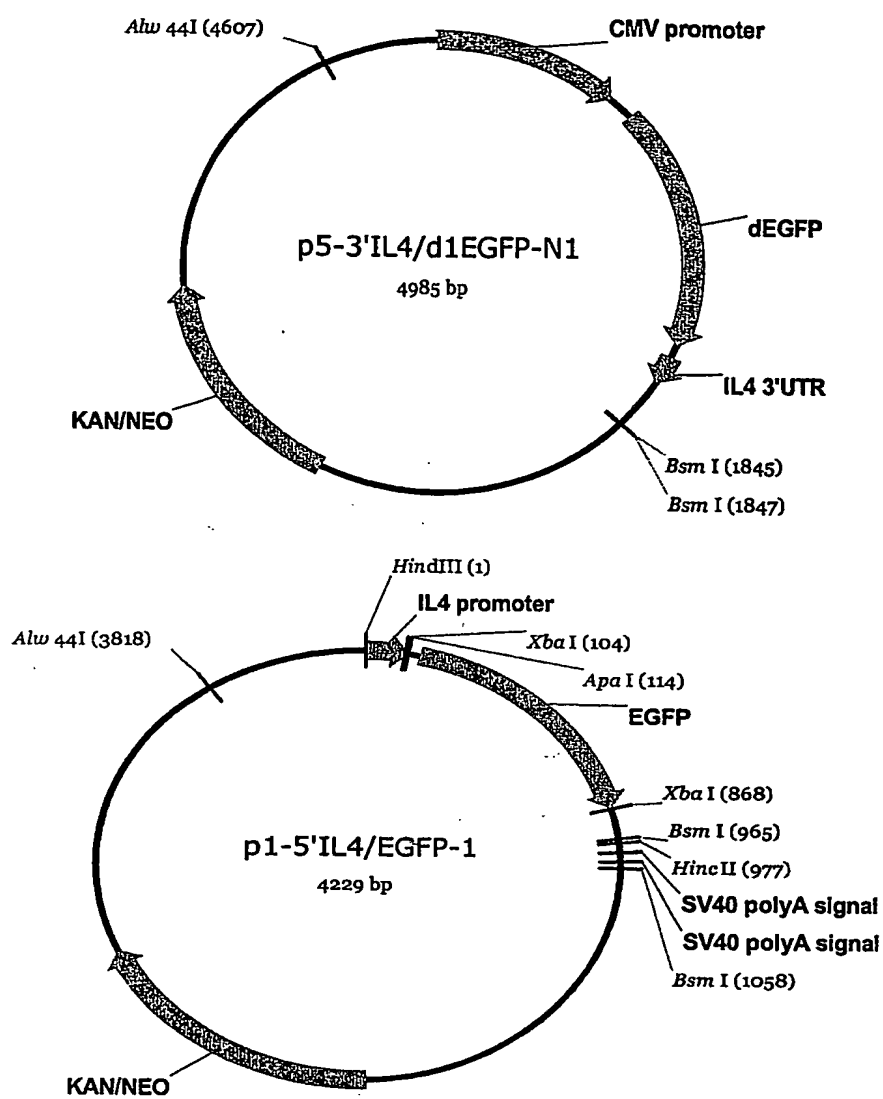


Fig. 12

**Fig. 13**

**Fig. 14**

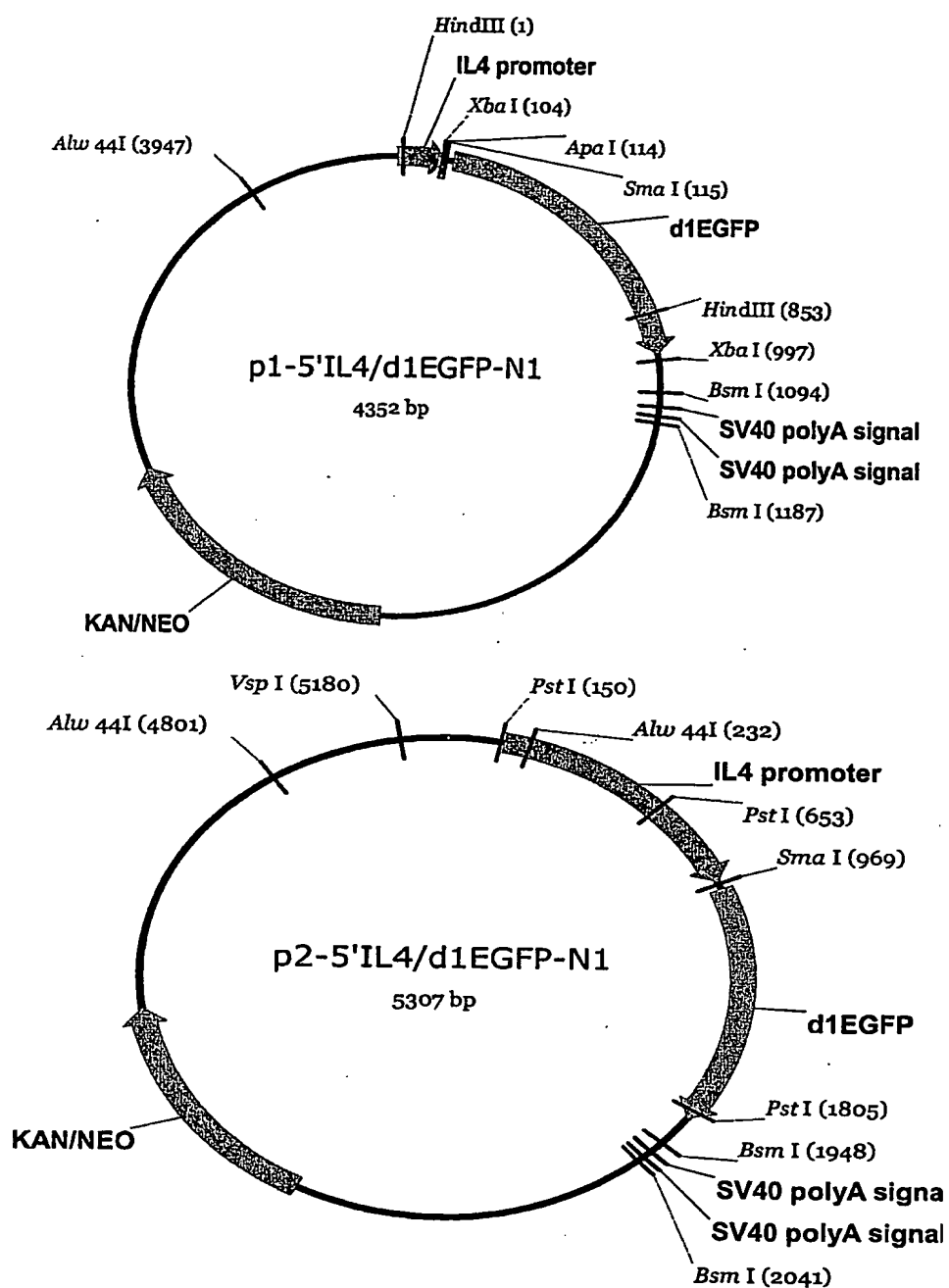


Fig. 15

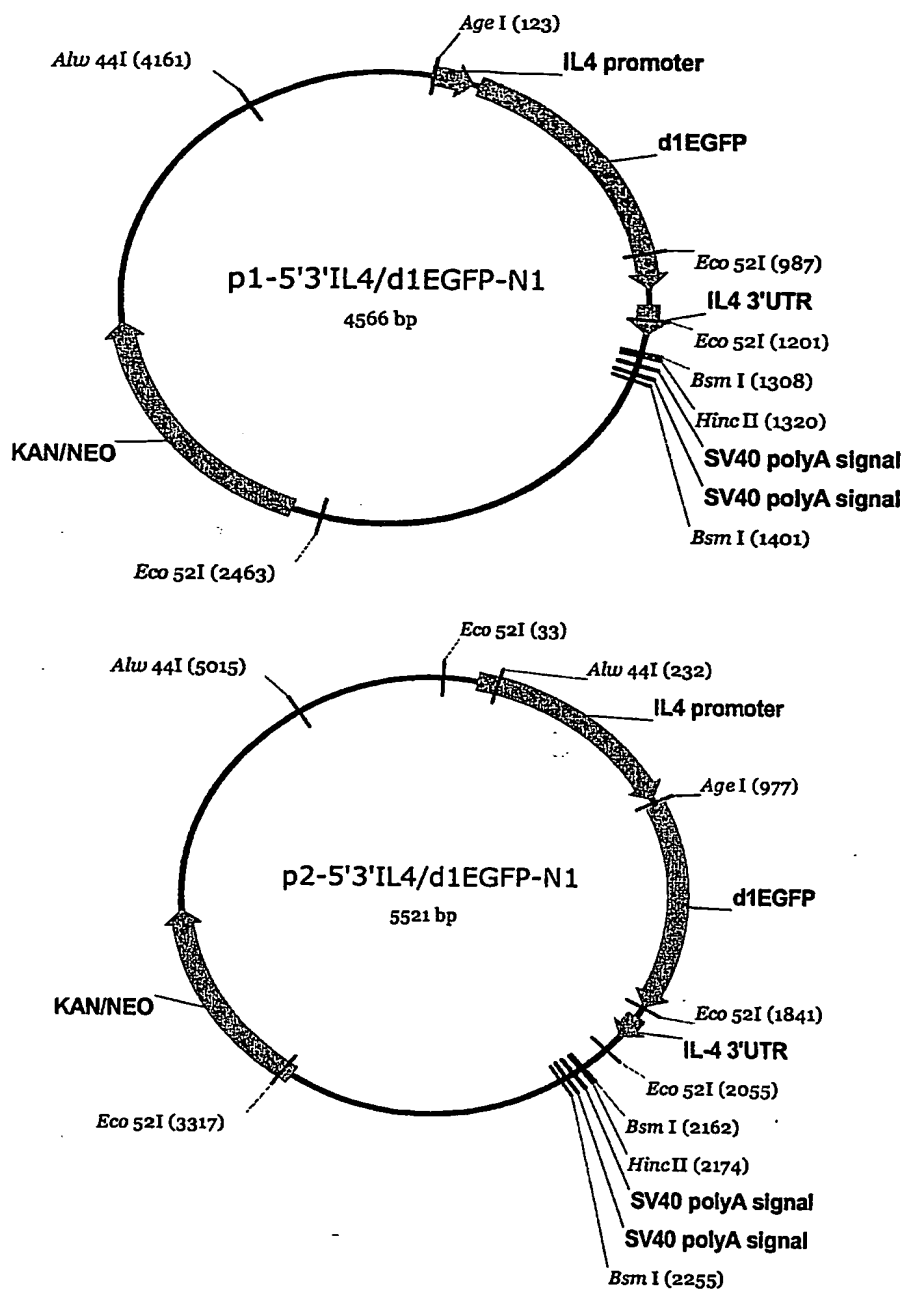


Fig. 16

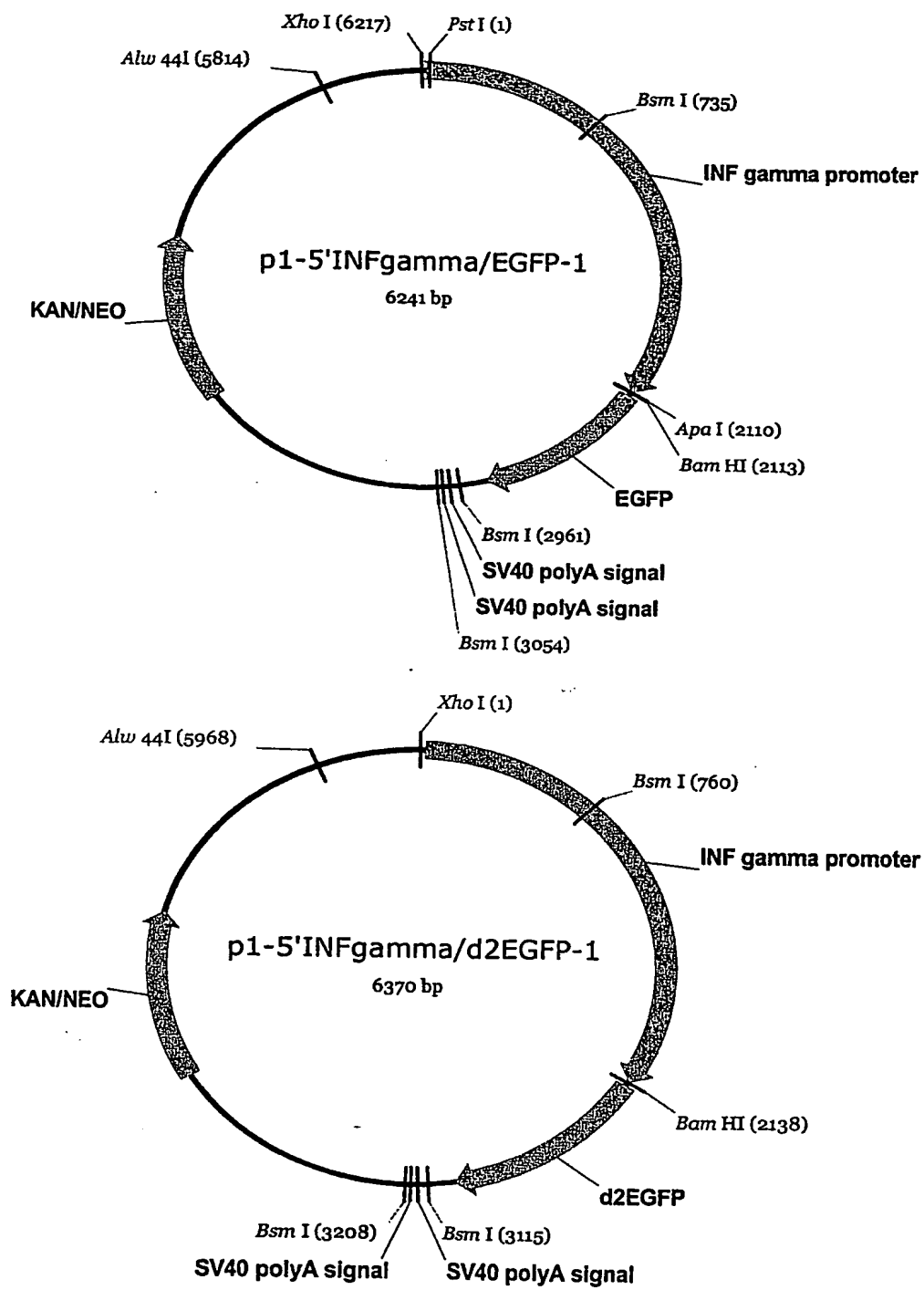


Fig. 17

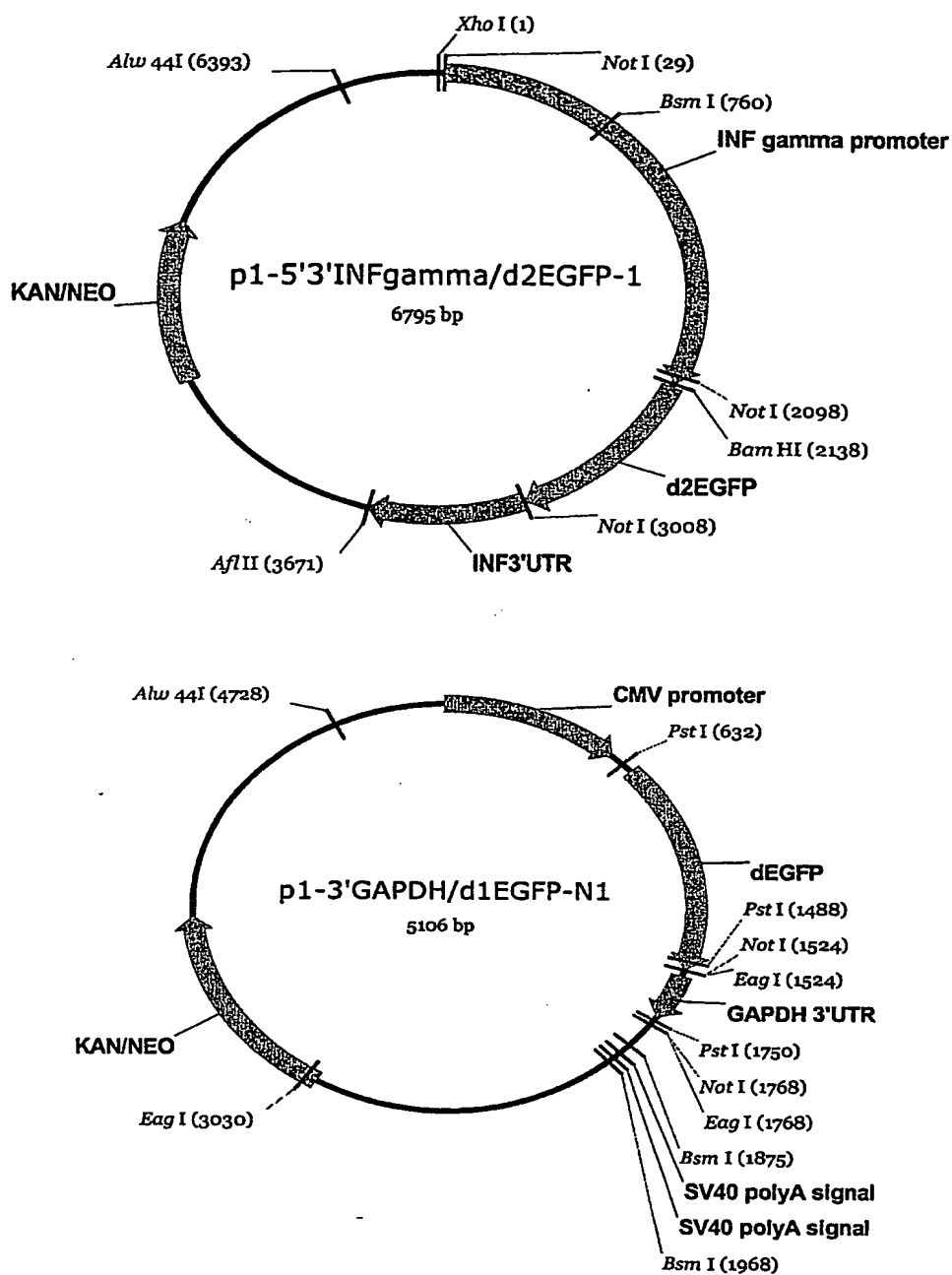


Fig. 18

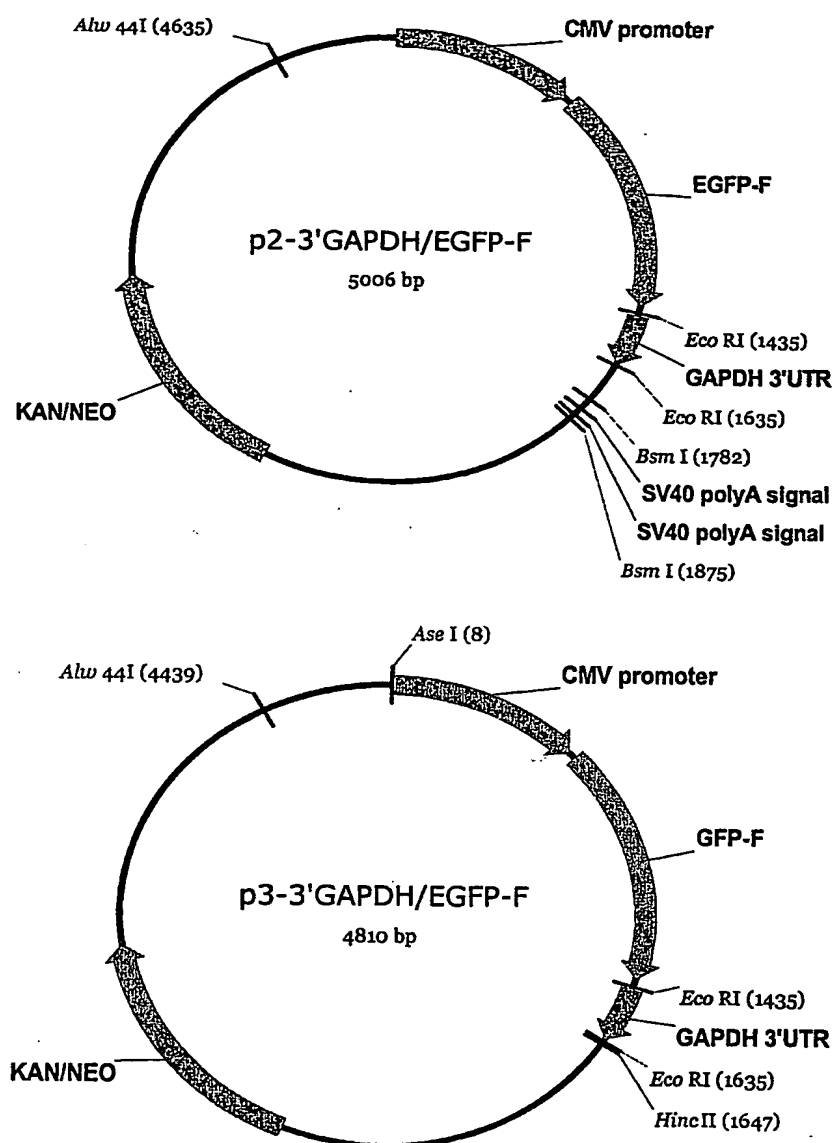


Fig. 19

**Fig. 20****Cell viability testing.**

Cell lines employed in the project were exposed to increasing concentrations of chemicals listed in Table II. LDH release was determined using test (Roche) according to the manufacturer protocol. All concentrations are expressed in  $\mu\text{M}$ .

Chemical	solvent	Hel.30 Karcinocyte	3T3-L1+pEGFP-F Fibroblast	EL-4 T-cell leukemia	J774A1 Macrophage- monocyte
Benzocaine CAS Nr: 94-09-7	Ethanol	A: - B: 3000, 1000, 100 C: 10 D: -	A: - B: 3000, 1000 C: 100, 10 D: -	A: - B: - C: 1500 D: 1000, 100	A: 3000 B: 1000 C: - D: 100, 10
Cyclosporin	Ethanol	A: - B: 10 C: 1, 0.1, 0.01 D: -	A: - B: 30, 10 C: 1, 0.1 D: -	A: 15, 10 B: 1 C: - D: -	A: 30, 10 B: - C: - D: 1, 0.1
DNCB, dinitrochlorobenzene CAS Nr: 9700-7	Ethanol	A: 100, 33, 10 B: - C: 3, 3 D: -	A: 100, 10, 3.3 B: - C: 1 D: -	A: 10 B: - C: - D: 1, 0.33, 0.033	A: 10, 5 B: - C: - D: 0.5, 0.05
MDI, diphenylmethane- 4,4-disocyanate CAS Nr: 101-68-8	DMSO	A: - B: - C: 1000, 100, 10, 1 D: -		A: 1500 B: - C: - D: 150, 15, 1.5	
HgCl <sub>2</sub> , mercuric chloride Cas nr: 7487-94-7	ethanol	A: - B: - C: 1, 0.1, 0.01, 0.001 D: -	X: 10 A: - B: - C: 1, 0.1, 0.01 D: -	X: 10 A: 30 B: - C: - D: 1, 0.01	

Fig. 20  
(continuation)

Chemical	solvent	Hel 30 Karcinocyte	3T3-L1+pEGFFP-F Fibroblast	EL-4 T-cell leukemia	J774A1 Macrophage- monocyte
Penicillin G Cas nr: 140-64-7	medium	A: - B: - C: 1000, 100, 10, 1 D: -	A: - B: - C: 1000, 100, 10, 1 D: -	A: - B: - C: - D: 1000, 100, 10, 1	
SDS, sodium dodecyl sulphate	DMSO	A: 500 B: - C: 50, 5, 0.5 D: -	A: 500 B: - C: 50, 5, 0.5 D: -	A: 250 B: - C: - D: 50, 5, 0.5	
TBTO, bis-tributyltin oxide Cas nr: 584-3-9	ethanol	A: 100, 10, 1 B: 0.1 C: - D: -	A: 100, 10, 1 B: - C: 0.1 D: -	A: 10, 1, 0.5, 0.1, 0.05 B: 0.1 C: - D: -	
TDI, toluene-2,4- diisocyanate Cas nr: 584-84-9	ethanol	A: 1500 B: 150 C: 15, 1.5 D: -		A: - B: 1000 C: - D: 100, 10, 1	
K2PtCl4, tetrachloroplatinate (platinum salt) Cas nr: 10025-99-7	medium	A: - B: - C: 8.6, 0.86, 0.086, 0.0086 D: -	A: 300, 100 B: - C: 10, 1 D: -	A: - B: 150, 50 C: - D: 5, 0.5	
Thalidomide, cas nr 50-35-1	DMSO	C: 1000, 100, 10, 1	C: 1000, 100, 10, 1	D: 1000, 100, 10, 1	

A: toxic concentration, B: concentration inhibits cell growth, no toxicity, C: no toxic effect or growth inhibition, X: lower amount of LDH, but no toxic effect or cell growth inhibition

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**Fig. 21****Comparison of data obtained in cytotoxicity tests in two participating laboratories**

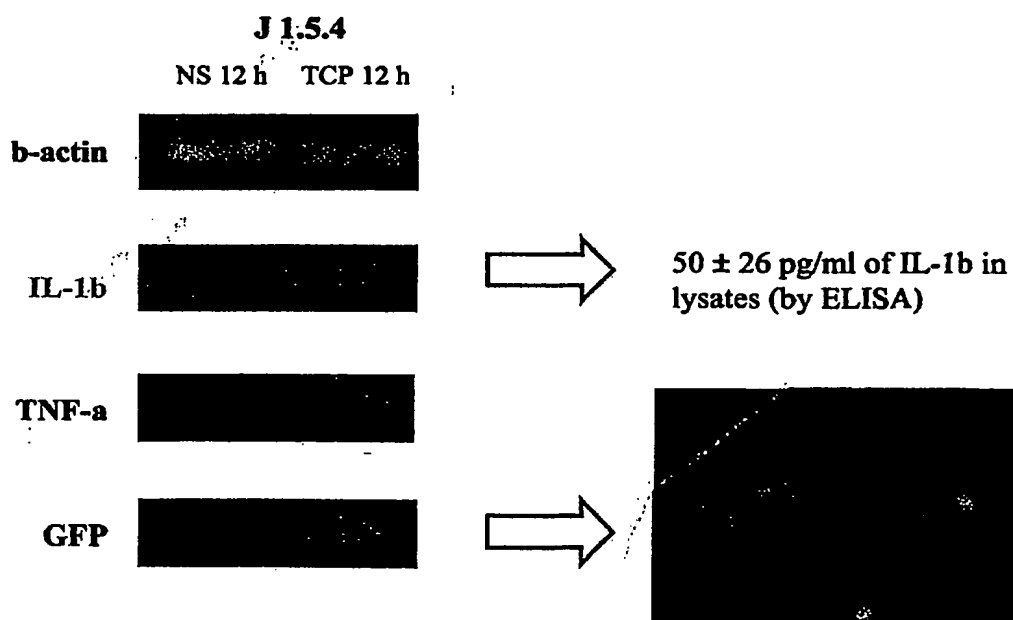
Direct cytotoxicity associated with chemicals listed in Table II was determined for EL-4 cell line using LDH release assay in two laboratories according to the same experimental protocol. All concentrations are expressed in  $\mu\text{M}$ .

Chemical	solvent	NIPH solv.	EL-4 T-cell leukemia	in NIPH
Cyclosporin	Ethanol	Ethanol	A: 15, 10 B: 1 C: - D: -	20ug/mL and above is Toxic below 10ug/mL OK
Penicillin G Cas nr: 140-64-7	medium		A: - B: - C: - D: 1000, 100, 10	2500 U = 15 mg/mL and below not toxic
Pentamidine cas nr: 140-64-7	medium	DMSO		30ug/mL and below=OK, 60ug/mL usure, 80ug/mL and above toxic
Rapamycin cas nr 53123-88-9		DMSO		Think 25000ng/mL toxic, below 1000 ng/mL OK
SDS, sodium dodecyl sulphate	DMSO	water	A: 1500 B: - C: - D: 150, 15, 1.5	50ug/mL and below not toxic, 2,5 mg/mL toxic
HgCl <sub>2</sub> , mercuric chloride Cas nr: 7487-94-7	ethanol		X: 10 A: 30 B: - C: - D: 1, 0.01	
TBTO, bis-tributyltin oxide Cas nr: 584-3-9	ethanol			
TDI, toluene-2,4-diisocyanate Cas nr: 584-84-9	ethanol			
K <sub>2</sub> PtCl <sub>4</sub> , tetrachloroplatinate (platinum salt) Cas nr: 10025-99-7	medium	water		150uM = - 20 % toxicity
Thalidomide, cas nr 50-35-1	DMSO			

**Fig. 22****Expression of IL-1 $\beta$  and GFP in J.1.5.4 stimulated with tetrachloroplatinate TCP**

A. The EC<sub>50</sub> values for selected chemicals from the list of model immunotoxicants (concentration causing death of 50% of cells in the population) obtained with MTT assay with macrophages J774A.1 and clone J 1.5.4.

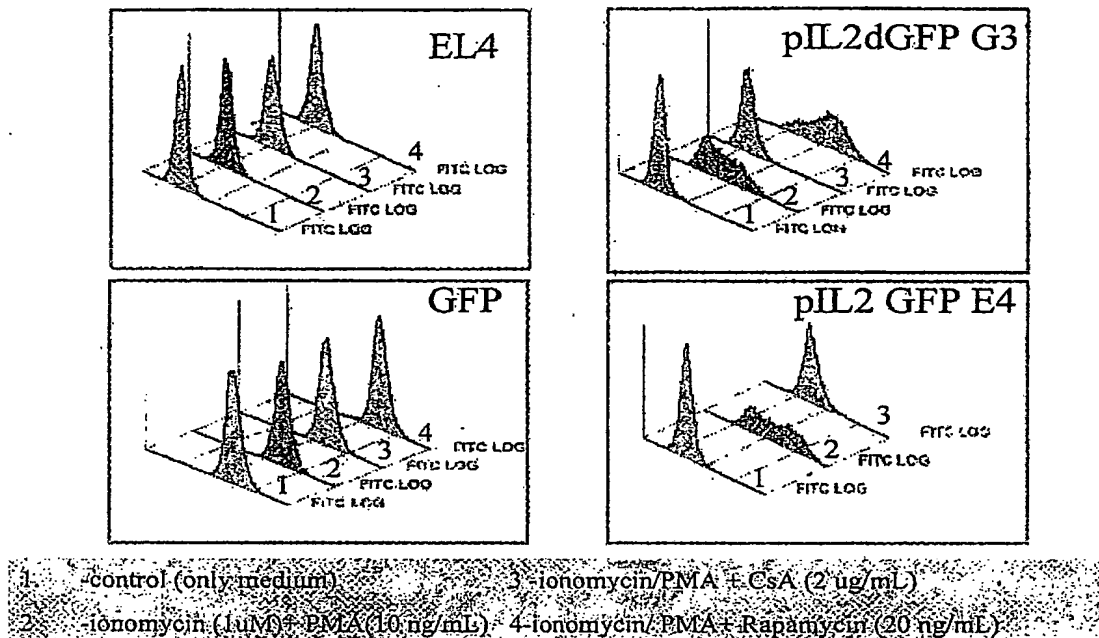
B. J 1.5.4. reporter cells were incubated with these chemicals and observed under fluorescence microscope. In the case of tetrachloroplatinate upregulation of green fluorescence was observed. The expression of GFP and endogenous IL-1 $\beta$  was confirmed with RT-PCR and with RT-PCR and ELISA, respectively.

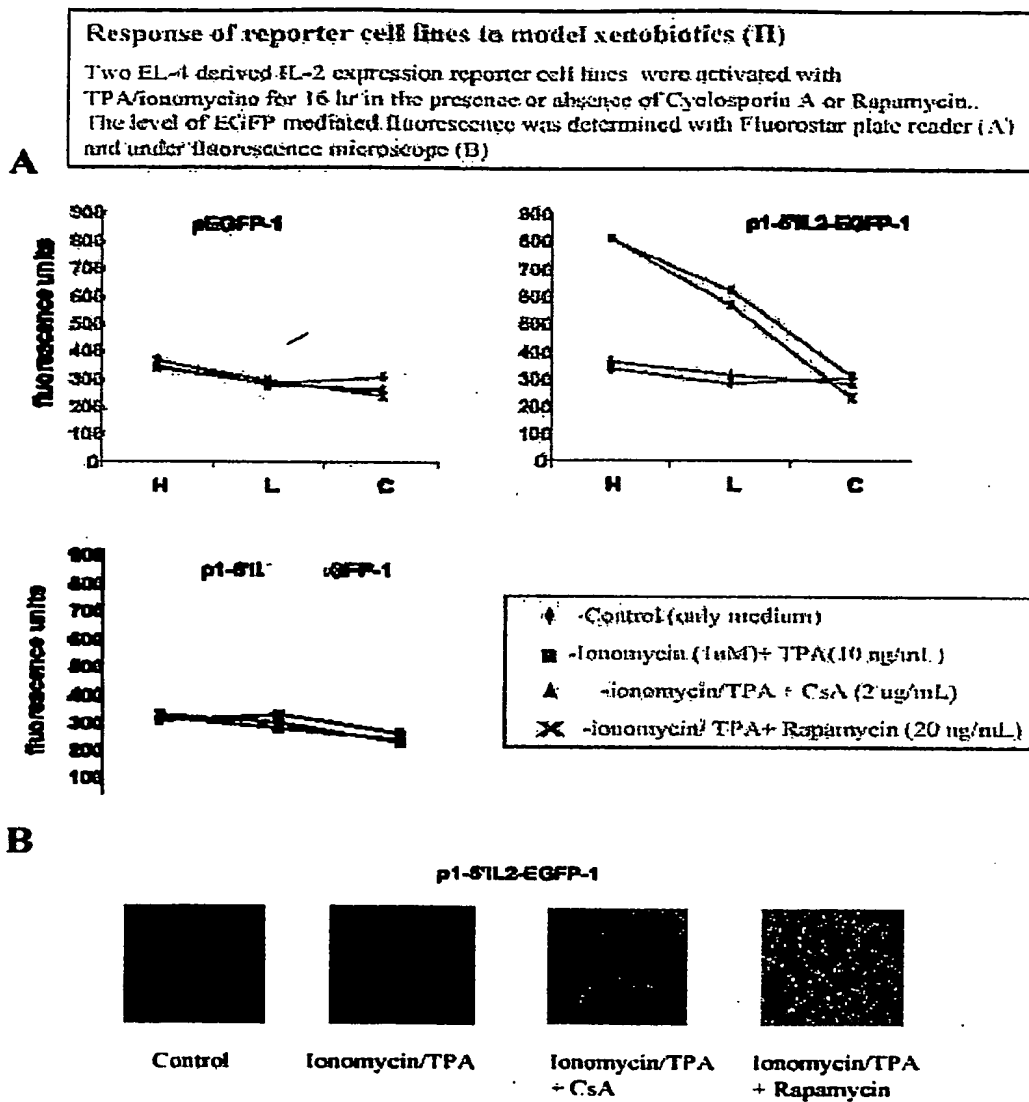


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**Response of reporter cell lines to model xenobiotics (I)**

Two EL-4 derived IL-2 expression reporter cell lines were activated with TPA/ionomycin for 16 hr in the presence or absence of cyclosporin A or Rapamycin. The level of EGFP mediated fluorescence was determined by FACS

**Fig. 23**

**Fig. 24**

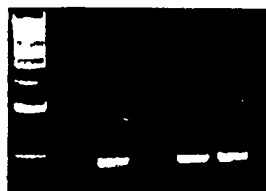
**Fig. 25****Response of reporter cell lines to model xenobiotics (III)**

A. EL-4 cells were incubated were activated with TPA/ionomycine for 16 hr in the presence or absence of Cyclosporin A or Rapamycin or TCDD. RNA was isolated using Tri reagent and RTPCR using primers specific for IL-2 and GAPDH (control) were performed. PCR products were analyzed on agarose gel

B EL-4 derived reporter cells were incubated with media alone or activated with TPA/ionomycine for 16 hr in the presence or absence of Cyclosporin A or Rapamycin for 16 hr and the level of EGFP mediated fluorescence was determined by FACS.

**A**

M 1 2 3 4 5



M: size marker

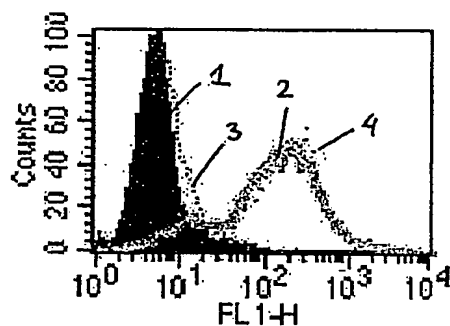
1: control

2: TPA/ionomycin

3: TPA/ionomycin + CsA

4: TPA/ionomycin + Rap.

5: TPA/ionomycin + TCDD

**B**

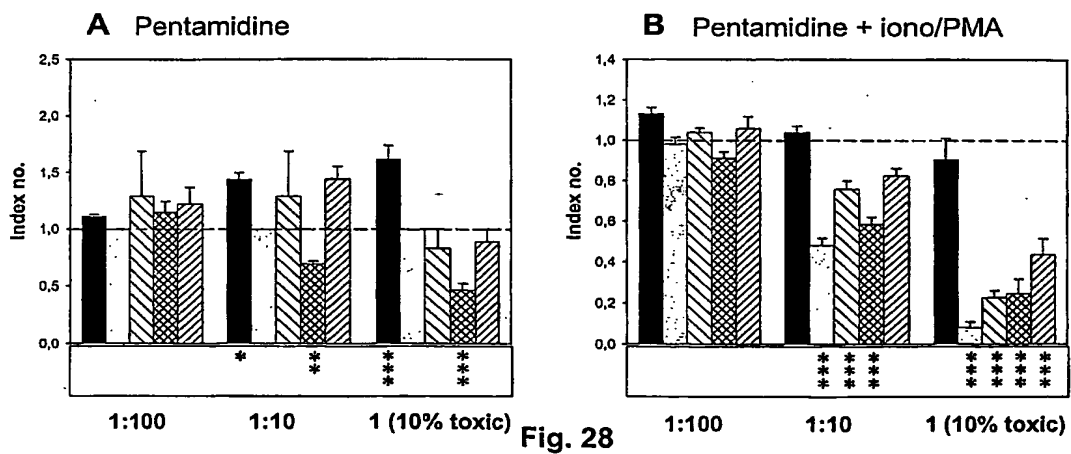
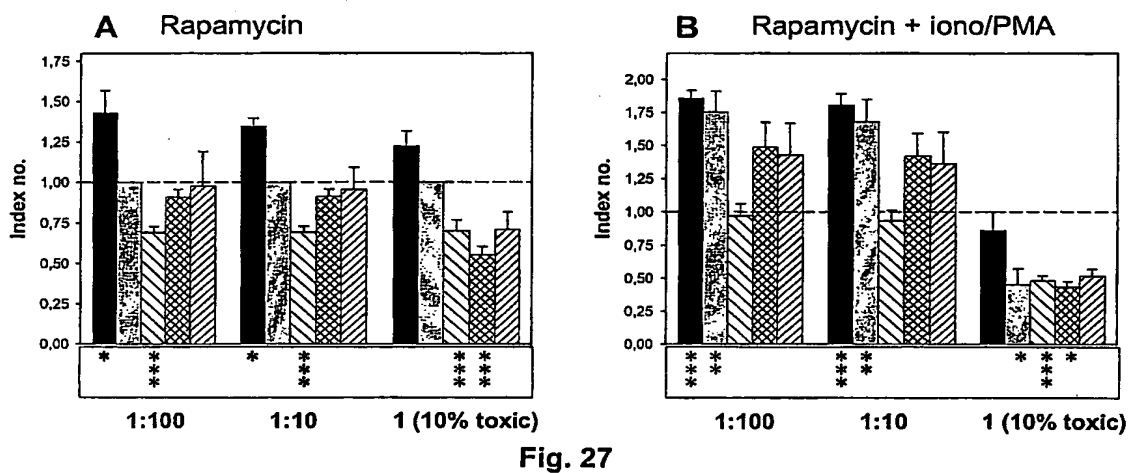
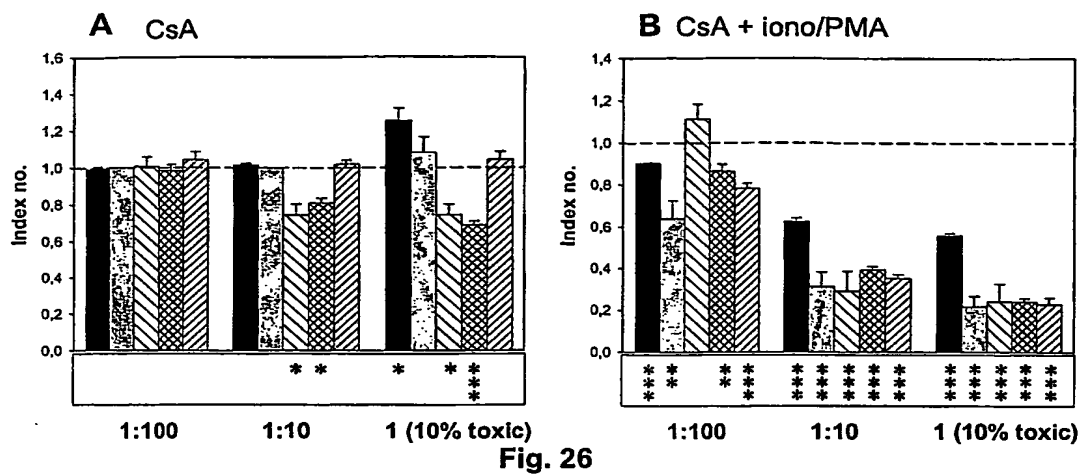
1 Control

2 TPA/ionomycin

3 TPA/ionomycin + CsA

4 TPA/ionomycin + Rap.

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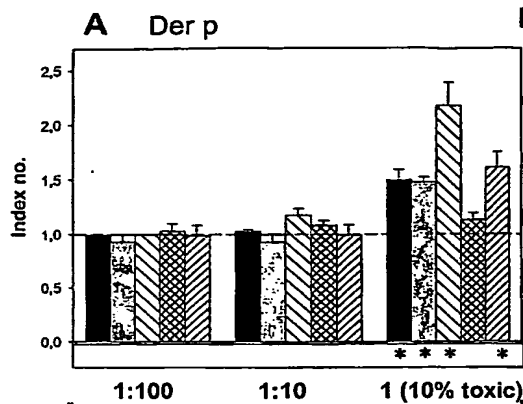


Fig. 29

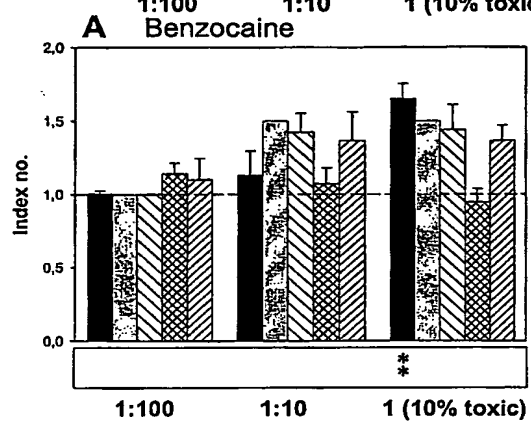
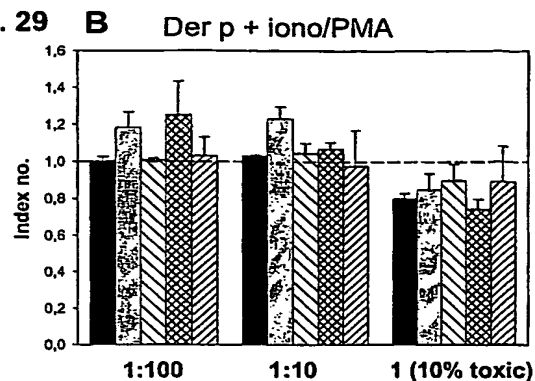


Fig. 30

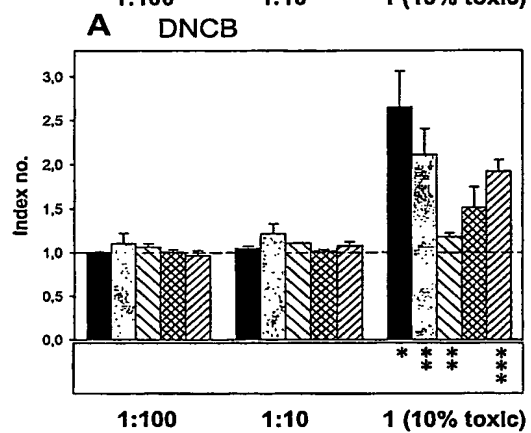
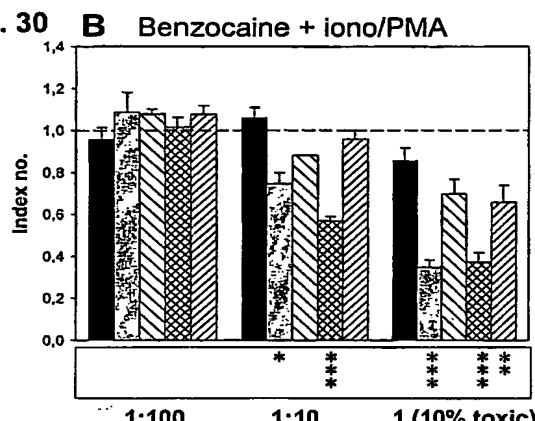


Fig. 31

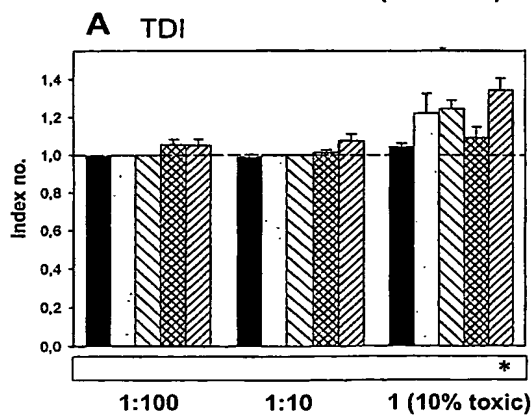
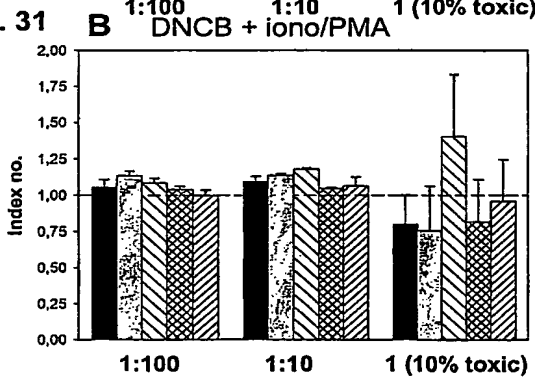


Fig. 32

